for the polarity profile along the bound PtdCho molecule and the one determined for membrane bilayers. This polarity match rationalizes the efficient energy-independent partitioning of a lipid molecule from a bilayer into the Sec14p phospholipid-binding pocket. Further, we have developed a direct method for observing formation of a hydrogen bond between sequestered water molecules and a spin-labeled site by applying pulsed Hyperfine Sub-level Correlation (HYSCORE) spectroscopy. Funded by: NSF-0843632 to TIS and NIH 1R01GM072897 to AIS.

3004-Pos Board B109

A Study of Traditional Chinese Medicine in Modulations of EGFR-EGF Interaction by Using Spr

Guo-Chung Dong.

Epidermal growth factor receptor (EGFR) is an important target on cancer therapy. One of the approaches is to modulate EGFR induced ligand-binding affinity change. In order to understand the correlation between ligand-binding change and EGFR modulation, we try to get some small molecular agents from traditional Chinese medicines (TCMs) to modulate EGFR activity on the biosensor system. Among 60 of TCMs, we find that some samples exhibit different influence on EGFR modulation. Among these, 051 showed a more inhibitive property due to decreasing EGF-affinity, -binding to EGFR and existed an anti-EGFR activity to A549 cells. On the other hand, 040 exist opposite function to 051. 040 is a most powerful enhancer that can increase the affinity and quantity of EGF binding to EGFR and a pro-EGFR activity on cell culture system. In our study, we can regulate the EGFR-mediated cell proliferation and anti-apoptosis by modulating the affinity of EGF-EGFR interaction. This way will be a useful method to apply on the therapy of human diseases.

3005-Pos Board B110

Targeting the NADPH Binding Site of Nitric Oxide Synthase by a Ligand with Two-Photon Absorption Properties

Etienne Henry, Yun Xu-Li, Huan Wang, Patrick Tauc, Jean-Luc Boucher, Anny Slama-Schwok, **Eric Deprez**.

Synthesis of nitric oxide is performed by NO-synthase. The catalysis is initiated by the combined transfer of electrons/proton from NADPH to the flavin FAD. We have previously characterized one photoactive compound (named Nanotrigger)1,2 allowing to trigger and synchronize NOS activity upon light illumination. Here, we described a new compound (C1) with two-photon absorption properties suitable to assess the binding to the NOS protein. This family of compounds combines a docking moiety (NADPH analog) and a chromophore moiety responsive for light illumination. C1 was characterized in DMSO by an absorption maximum at 460nm and was found to be fluorescent with an emission peak centered at 740nm upon one-photon excitation. However, the fluorescence emission was strongly sensitive to solvent polarity as evidenced by the significant decrease in the emission intensity in polar solvents. The same behaviour was observed under two-photon excitation (Exc, 940nm). One- and two-photon fluorescence approaches were used to assess the binding of C1 to the neuronal NOS. Under one-photon excitation, the emission properties of the complex were rather difficult to interpret due to the overlapping between the intrinsic fluorescence of nNOS and C1 fluorescence. As the two-photon fluorescence of nNOS protein is very weak, the complex formation was measured by monitoring the two-photon fluorescence recovery of C1 upon binding to nNOS. The calculated Kd value was found to be consistent with the value characterizing NADPH binding. However, competition experiments suggest that the competition between C1 and NADPH is more complex than expected from a one-binding site model. Our results suggest that C1 represents a promising compound for cellular applications in two-photon fluorescence imaging.

1 Beaumont et al., J. Am. Chem. Soc. 2007, 129, 2178-2186.
2 Beaumont et al., ChemBioChem 2009, 10, 690-701.

3006-Pos Board B111

Fluorescent Binding Studies of Phosphofructokinase from Bacillus Stearothermophilus Using a Tryptophan-Shifted Mutant

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Phosphofructokinase from Bacillus stearothermophilus (BsPFK) is an allosteric, homotetrameric enzyme containing one tryptophan per subunit. Unlike the homologous PFK from E. coli (EcPFK), the fluorescence of the native tryptophan is unresponsive to ligand binding. This study utilizes a tryptophan-shifted mutant, in which W179 has been mutated to phenylalanine, and F240 has been mutated to tryptophan. The variant is functionally similar to wild-type, however a decrease in the fluorescence of 6.5% is associated with substrate fructose 6-phosphate (Fru-6-P) binding and a decrease of 16% is associated with inhibitor PEP binding. Dissociation constants of $1.9 \pm 0.3\mu M$ for Fru-6-P and $107 \pm 13\mu M$ for PEP were thereby determined. This dissociation constant for PEP is in good agreement with that determined by kinetic assays ($128 \pm 5\mu M$). Due to the absence of known ATP antagonism, the dissociation constant for Fru-6-P in the absence of MgATP is lower than with steady-state kinetic assays determined at saturating MgATP ($36 \pm 1\mu M$). The coupling between PEP and Fru-6-P has also been determined and increases with temperature as observed with steady-state kinetic assays using wild-type BsPFK. Like wild-type PFK, the coupling free energy results from compensating enthalpy and entropy components. The sign of the coupling free energy is opposite that of the enthalpy and is therefore determined by the larger absolute value of the entropy term. This is opposite the thermodynamic basis of the allosteric response in EcPFK, where the sign is established by the enthalpy component. The functional and thermodynamic similarity of the mutant enzyme to wild-type, together with the added ability to follow ligand binding with fluorescence, make it a good candidate for further studies probing a deeper understanding of the thermodynamics involved in PFK allosterism. Funding provided by the following: NIH-GM33216, NIH-CBI, and the Welch Foundation.

3007-Pos Board B112

Characterizing Riboflavin Antagonists for Targeted Drug Delivery Applications

Anna Plantinga, Amanda Witte, Seok-Ki Choi, Kumar Sinniah.

Riboflavin ligands present an alternative pathway for targeted drug delivery as riboflavin receptors are over-expressed in breast and prostate cancer cells. We have examined riboflavin and several riboflavin mimicking molecules (antagonists) for targeting the riboflavin binding protein, which acts as a model protein for the riboflavin receptor. Isothermal titration calorimetry (ITC) was used for determining the binding constant of riboflavin (RF) and RF antagonists to chicken riboflavin binding protein (RfBP). The equilibrium dissociation constants determined for riboflavin (Kd = 1.4 nM) and lumiflavin (Kd = 64.2 nM) in 0.1 M phosphate buffer (pH 7.4) by the microcalorimetric method are in close agreement with the binding data previously determined by fluorescence quenching spectrometry. Several of the RF antagonists screened showed dissociation constants in the micromolar range while some exhibited no binding. The RF antagonists will be used in future studies to target riboflavin receptors for cellular uptake as a potential route for the selective delivery of drug molecules to cancer cells that over-express riboflavin receptors.

3008-Pos Board B113

Aluminum Phosphate Adsorption of Proteins Using Isothermal Titration Calorimetry

Ronan O'Brien, **Verna Frasca**, Mark Arsenault, Mary Jo Wojtusik.

Isothermal titration calorimetry (ITC) is widely used for measuring the affinity and thermodynamics of biomolecular interactions. These instruments measure the 10^{-7}°C change in temperature that occurs when micromolar concentrations of biological molecules interact in solution. Recently it has become apparent that the technique can also be used for studying heterogeneous systems such as the interaction between proteins in solution and the largely insoluble aluminum phosphate, the only FDA approved adjuvant used in vaccine formulations. Here we will describe results of measuring the adsorption of a number of proteins to aluminum phosphate, using ITC, highlighting the possible applications in vaccine development.

3009-Pos Board B114

Characterizing the Interaction Between Phthalocyanine Tetrasulfonates and Mammalian Prion Protein

Iveta Sosova, Abhilash Vincent, Amarnath Gupta, Max Anikovskiy, Angela Brigley, Michael T. Woodside.

Phthalocyanine tetrasulfonates are known to interact with mammalian prion protein, having the ability to act as anti-prion agents and help prevent the conversion of native isoform PrPc to the scrapie isoform PrPsc. However, the interaction between phthalocyanines and PrP remains poorly characterized. We explore the phenomenology of this interaction in detail by examining the binding of phthalocyanines to monomeric native Syrian Hamster prion protein using multiple complementary assays: surface plasmon resonance, FCS, fluorescence quenching, calorimetry, and CD spectroscopy. We determine binding constants, kinetics, stoichiometry, structural and spectroscopic effects of binding, and the influence of buffer ionic strength.